LIFE CYCLE OF THE TREMATODE, DIPLOSTOMUM MURRAYENSE J. & C.

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In 1938 was published an account of *Cercaria murrayensis*, a common furcocercaria occuring in *Limnaea lessoni* in the swamps of the Lower Murray River (Swan Reach and Tailem Bend), infection being observed from December to May, the infection rate varying from 6 to nearly 50% (Johnston and Cleland, 1938). Its similarity to *C. flexicauda* Cort and Brooks from North America was noted. The parasite was allotted to the *Proalaria* group (*Proalaria* is now considered a synonym of *Diplostomum*), and its next larval stage, a *Diplostomulum*, was stated to occur in the eyes of freshwater fish. The sporocyst stage was also described.

Next year an account was given of the metacercaria, *Diplostomulum murrayense*, obtained from the lens of various species of fish in about six weeks after experimental infections with cercariae taken from October to April. The infection route was traced and found to be similar to that described by Van Haitsma (1931) for *D. flexicaudum*. A review of the literature relating to the occurrence of similar parasites in the eyes of freshwater fish in Europe and North America was also given. These parasites were stated to be of considerable economic importance because heavy infection, in the case of very young or small fish, commonly resulted in high mortality (Johnston and Simpson 1939).

Freshwater fish reported capable of being infected experimentally with *C. murrayensis* were golden carp (*Carassius auratus*), rice fish (*Oryzias latipes*), congolli (*Pseudaphritis urvillei*), *Pseudomugil signifer* and *Melanotaenia nigrans*, the last three being native fish. Natural infection was reported to have been observed in the lens of larger specimens of the golden carp, Murray cod (*Maccullochella macquariae*), callop (*Plectroplites ambiguus*), and Murray bream (*Therapon bidyana*), all from Tailem Bend. Attempts to obtain the adult stage by feeding diplostomula to laboratory-bred white rats and to muscovy ducklings led to negative results. It was believed that the adult would be found in gulls or terns; most probably the silver gull, *Larus novaehollandiae*.

Later experience led us to regard the marsh tern, *Chlidonias leucopareia*, as the probable host, because of abundance of that bird on the swamps from late spring to autumn, its food consisting mainly of dragonfly larvae, prawns and small fish. Thanks to the assistance of Messrs. G. and F. Jaensch and L. Ellis, we were able to examine some of these birds, finding minute diplostomes in four out of nine of them, taken during the period November to March, some of the parasites being very young and similar in size and anatomy to the parasites from fish eyes. On one occasion fish lenses were also present in the digestive tract, and, on another, abundant remains of very small fish were seen along with various stages in the development of the trematode from the diplostomulum stage to the adult.

Since the original account was published, we have found in twelve collections of *Limnaea lessoni* taken at Tailem Bend during the summer months 1938-41, infection varying from 0 to 25%, *C. murrayensis* being identified from 68 out of 680 snails, *i.e.*, in 10% of the total examined during the period.

Eggs from adult diplostomes taken from a marsh last December were added to a small aquarium containing laboratory-bred *L. lessoni*, cercariae (*C. murrayensis*) being noticed 36 days later. Fish (*Gambusia affinis*) were subjected to infection by these cercariae, many fully developed diplostomula (fig. 5) being recovered from the lenses four weeks after the earliest infection; hence the minimum period may be less than that observed. Daily attempts were made to infect tadpoles of *Limnodynastes tasmaniensis*, but this seems to be a refractory host, since the parasites found in the lens had not passed beyond the tail-less cercaria stage even after six days from the commencement of infection.

Since the original account of the diplostomulum stage was published, we have found it occurring under natural conditions during the summer 1940-41 in the following fish in the Murray River or swamps at Tailem Bend and Murray

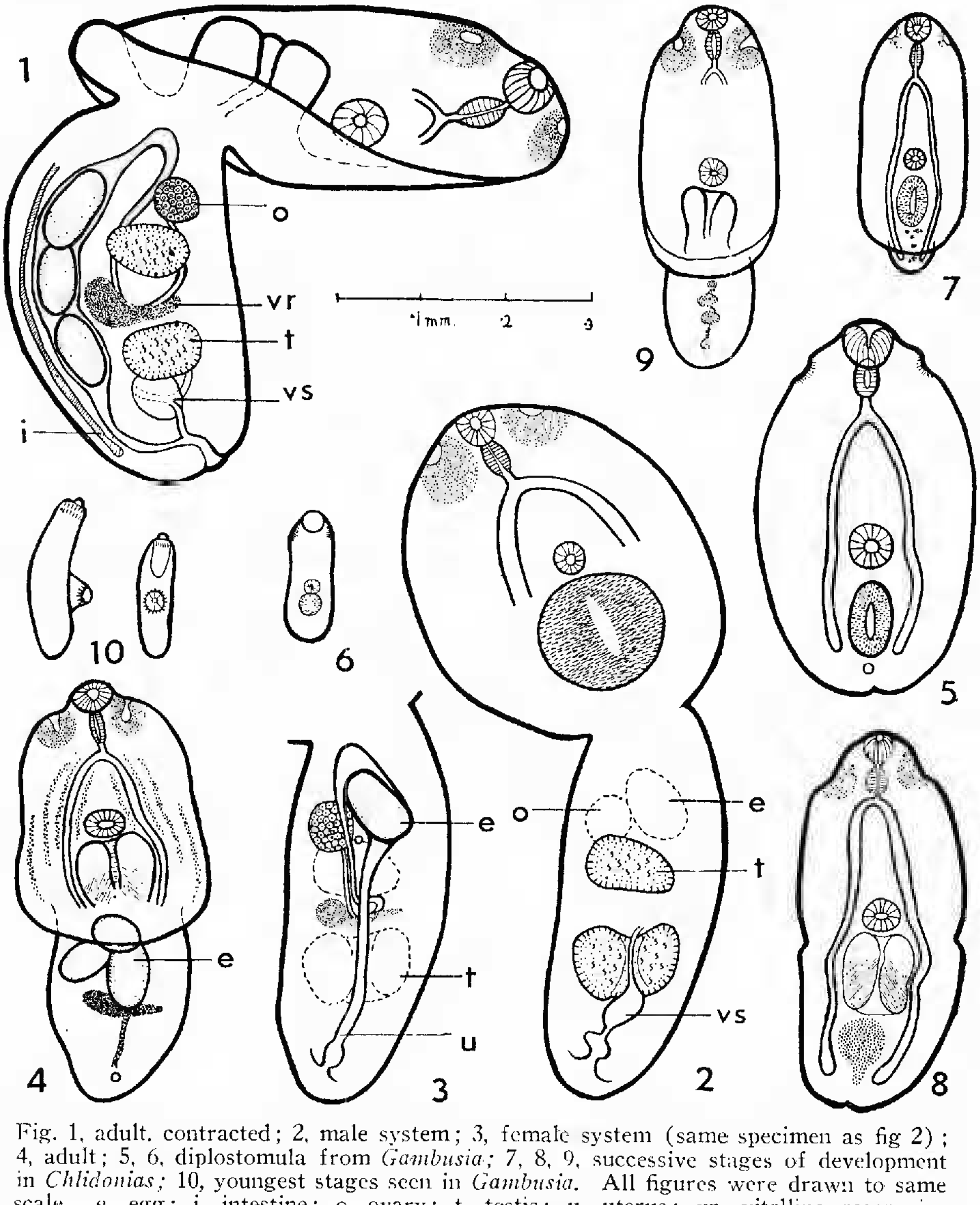

Fig. 1, adult, contracted; 2, male system; 3, female system (same specimen as fig 2); 4, adult; 5, 6, diplostomula from *Gambusia;* 7, 8, 9, successive stages of development in *Chlidonias;* 10, youngest stages seen in *Gambusia*. All figures were drawn to same scale. e, egg; i, intestine; o, ovary; t, testis; u, uterus; vr, vitelline reservoir; vs, vesicula seminalis.

Bridge: *Retropinna semoni*, *Carassiops klunzingeri*, *Melanotaenia nigrans*, *Nannoperca australis*, *Pseudaphritis urvillei*, *Craterocephalus fluviatilis*, *Mugilogobius galwayi*, *Philypnodon grandiceps*, *Mogurnda adspersa*, *Percalates colonorum* and *Carassius auratus;* and from *Galaxias attenuatus* and *G. olidus* from other South Australian localities. Our records indicate that *Cercaria murrayensis* has been observed each month from November (rarely October) to

May inclusive, and that the diplostomulum stage has been collected from the lens of fish in the same locality (Lower Murray) each month from November to May inclusive, but not in those taken in June, August and October. These observations indicate that snails (unless the infection has survived the winter) may become infected in September or October by eggs which have passed through the winter in the swamp or which have been present in the faeces of the earliest terns to arrive in the spring. By October cercariae have become available to infect fish in which fully developed diplostomula may be present in November when the terns may become infected. Our original observation that infection, if present, was always light in the case of large fish, can be explained by the habit of such fish, since they rarely visit the shallow swamps (where small species and the young of all species are to be found), but live in the river and deeper channels where the food plants on which *Limnaea lessoni* feeds, do not find a suitable environment for their growth.

We have now recorded the occurrence of the diplostomulum stage in the lens of fifteen species of native freshwater fish and one introduced species from South Australia, as well as (experimentally) from two exotic fish (*Oryzias* and *Gambusia*) commonly kept in aquaria. The extensive range of the species of fish concerned and the wide variety of orders and families involved suggest that the parasite may be expected to be able to infect additional species. Dubois (1938, 192) listed over forty species of freshwater fish from the northern hemisphere recorded by various observers as hosts for the diplostomulum stage of an allied *Diplostomum* (*D. spathaceum*) which occurs in gulls in Europe and North America.

The length of egg-bearing specimens of *Diplostomum murrayense*, lying flat in fluid, without compression, ranged from ·5 to 1 mm. The total length of nine such worms, the length and breadth of the fore- and hind-body respectively, and the approximate ratio of the length of the post-body to the fore-body, were as follows: (1) ·5 mm., (·28 × ·22 + ·22 × ·154), 1:·78; (2) ·5 mm., (·26 × ·24 + ·24 × ·16), 1:·9; (3) ·54 mm. (·25 × ·19 + ·29 × ·13), 1:1·16; (4) ·67 mm., (·36 × ·36 + ·31 × ·18), 1:·86; (5) ·67 mm., (·33 × ·28 + ·34 × ·23), 1:1; (6) ·85 mm., (·4 × ·35 + ·45 × ·19), 1:1·12; (7) ·88 mm., (·47 × ·43 + ·41 × ·29), 1:·87; (8) ·89 mm., (·53 × ·3 + ·36 × ·21), 1:·68; (9) 1 mm. (·5 × ·42 + ·5 × ·23), 1:1. Most adults seen were ·5 to ·7 mm., few between ·7 and ·9, and extremely few measured 1 mm. in length. The breadths and relative lengths of the two parts of the body varied, but the post-body was usually approximately equal to, or slightly greater than, the fore-body in length.

A few strongly contracted specimens with the hind-body lying at right angles to the fore-body were also measured (seen in lateral view) in fluid: (1) estimated total length ·84 mm., fore-body (including its posterior region projecting beyond the hind-body) ·55 mm. long, hind-body ·55 mm. with a maximum dorso-ventral diameter ·26 mm.; (2) ·80 mm., ·67 mm., ·3 (markedly arched ventrally), and ·34 respectively; (3) ·88, ·65 (with depth ·2 mm.), ·42 and ·32 mm. respectively.

Anterior sucker ·04-·06 mm. diameter, rounded or slightly longer than wide. Ventral sucker about same size, outline circular or slightly broader than long, anterior border (in fully adult specimens) distant from the anterior end of the worm 44 to 47%, occasionally 50%, of length of fore-body. Anterior glands each between ·05 and ·06 mm. long, with definite cavity directed antero-laterally or almost forwards. Holdfast (tribocytic) organ about ·1-·12 mm. long, about ·1-·15 broad, sometimes round; projecting prominently (especially in strongly contracted worms); often overlapping part of ventral sucker, but more usually a slight interval between the two organs; opening slightly longitudinal, with short groove leading into a canal surrounded by deeply-staining tribocytic glands;

breadth of organ about ·43 mm. or less, under half maximum breadth of fore-body. Genital pore dorsal, about ·04-·05 mm. from posterior end; excretory pore a short distance behind it.

Anterior sucker directed more or less ventrally; prepharynx very short, ·01 mm. long, above end of oral sucker; pharynx elongate, ·04-·06 mm. long, ·02-·03 mm. wide; oesophagus short, ·01 mm. long; caeca extending back close to base of tribocytic organ and almost reaching end of worm.

The reproductive system has the same general disposition as in *D. flexicaudum, D. spathaceum, D. huronense* and *D. indistinctum*. The testes have the form usual in the genus. The front of the anterior testis lies at about ·13 mm. behind the junction of the fore- and hind-body (*i.e.*, at less than 30% of the length of the hind-body), and adjacent to, or partly above, the posterior edge of the ovary. Its length is about ·05 mm., and the breadth ·1 mm. It is longer directly behind the ovary and tapering somewhat towards the opposite side of the body. The second testis, measuring ·07 by ·13 mm., is curved in transverse section, with its limbs directed ventrally, and with its front edge just behind (55-64%) half the total length of the post-body. The vasa deferentia were not traced fully but they pass back between the limbs of the second testis. The large rounded vesicula seminalis lies just behind, and partly below, the arch of the second testis. The narrow ejaculatory duct enters the genital atrium above the uterus.

The spherical ovary, ·05 mm. diameter, lies dorsally, immediately in front of the first testis and may be partly overlapped by it. The anterior end of the organ is at about ·07 mm. (at 15% of the total length of the hind-body). The oviduct travels back above part of the anterior testis to pass through Mehlis's gland lying dorsally between the two testes. The yolk reservoir is ventral and transversely placed, entering the ootyp from below. The uterine duct passes downwards and curves so as to lie antero-ventrally from, and parallel with, the ootyp, and then forwards as the ascending uterus below the anterior testis, extending into the region of the junction of the fore- and hind-body. It then curves back, traversing the ventral region below the testes and ootyp to reach the genital atrium. Laurer's canal is short and opens dorsally between the ovary and the anterior testis. Yolk glands extend forwards into the region just in front of the ventral sucker. The follicles obscure most of the organs in the hind-body, except in the vicinity of the genital atrium. There are 1 to 12 large eggs, usually 3 or 4, in the uterus; they measure ·072-·1 mm. by ·04-·06 mm., generally ·09 by ·06.

D. murrayense differs from *D. flexicaudum* as figured by Van Haitsma (1931), and *D. spathaceum* as figured by Krause (1914), Fuhrmann (1928), and Dubois (1938), in its dimensions, body ratios, position of the ovary and number of eggs. It closely resembles *D. huronense* La Rue (1927) and especially *D. indistinctum* Guberlet (1923, syn. *D. confusum* Gub. 1922) in the disposition of its organs, but differs in the detailed measurements of them and particularly in the size of the worms.

Some very young stages of the parasite were recovered from two marsh terns. For comparison with the youngest obtained, we mention the dimensions (in μ) of the diplostomulum stage (killed with boiling formalin) as given in the original account: body length 231-392 (mean 296); breadth 154-215 (177); anterior sucker 22-43 (34) long by 42-51 (47) broad; ventral sucker 30-37 (34) long by 34-47 (39) broad; holdfast 71 long by 79 broad. The largest specimens we obtained from *Gambusia* were rather larger than those described but were somewhat swollen by postmortem changes in the dead fish before we found them, the normal dimensions having probably become slightly increased—measurements in mm.: ·4-·44 long, ·2-·25 broad; anterior sucker ·05 by ·05-·06; ventral sucker ·05 diameter; holdfast ·09 by ·06 and ·07 by ·07. The smallest found in the bird host measured ·3 mm. long by ·12, with fore-body ·28 long and a minute post-body ·02 long by ·05; anterior sucker ·035 by ·03; ventral sucker ·035 by ·025;

glandular areas (head) ·015-·02 long; holdfast ·04 by ·018 (grooved portion), ·06 by ·04 if glandular region be included; pharynx ·025 by ·013; oesophagus ·01 long; genital anlagen represented by relatively few deeply-staining cells in the fore-body behind the holdfast and by cells in the post-body indicating the differentiating genital ducts and pore. Another was slightly larger but did not exhibit any differentiation into fore- and post-body. Its dimensions were: ·45 mm. long, ·19 broad; anterior sucker ·04 by ·03; posterior sucker ·035 by ·04; glandular areas ·055; holdfast ·09 broad; pharynx ·03 by ·02; genital anlage pyriform, much larger than in the preceding specimen but not differentiated, situated in the posterior ·12 mm. behind the deeply-staining paired holdfast glands. In its characters it resembled closely the diplostomulum stage. Another young worm, ·44 mm. long, possessed the following features: fore-body ·3 by ·16; post-body ·14 by ·1; anterior sucker ·03 by ·035; ventral sucker ·03 by ·03; holdfast prominent, ·06 by ·06, with well marked groove and two deeply staining glandular masses; pharynx ·03 by ·02; genital anlagen in three masses representing ovary, anterior testis and posterior testis, also a cord of cells representing the terminal portions of the differentiating uterus and seminal vesicle. A specimen ·4 mm. long, with fore-body ·3 by ·19 and hind-body ·1 by ·11, exhibited a similar stage of reproductive development as seen in the preceding worm. Yolk glands seem to be fully differentiated before the sex organs become functional, the latter occurring when the parasites have become about ·5 mm. in length.

In addition to the diplostomula, already referred to above, taken from *Gambusia,* we obtained from the same fish several very early stages, also from the lens. Some of these were practically tail-less cercariae (fig. 10) measuring from ·14 by ·04 to ·19 by ·05, with the anterior organ still persisting and with rows of spines around the head end, as well as a prominent row surrounding the projecting ventral sucker. A minute diplostomulum (fig. 6) was also obtained, measuring ·15 mm. long, ·05 broad; with an anterior sucker ·02 mm. long by ·024 mm.; a posterior sucker ·02 by ·02 mm.; a tribocytic organ immediately behind the latter and provided with a deep groove; and head glands like those of older diplostomula; but all spines had disappeared from the body and from the ventral sucker. These various stages from *Gambusia* were obtained as a result of submitting the fish to infection at short intervals by a very small number of cercariae.

Diplostomum murrayense is the first Australian Strigeate trematode whose complete life cycle is known, and is the first member of the genus, as now restricted, to be described from the Commonwealth.

Type material of the various stages is deposited in the South Australian Museum, Adelaide. Acknowledgment is made of the generous assistance rendered by Messrs. G. and F. Jaensch and L. Ellis of Tailem Bend in regard to material; and by the Commonwealth Research Grant to the University of Adelaide.

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